Hierarchical and Cybernetic Nature of Biologic Systems and Their Relevance to Homeostatic Adaptation to Low-level Exposures to Oxidative Stress-inducing Agents

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During evolution in an aerobic environment, multicellular organisms survived by adaptive responses to both the endogenous oxidative metabolism in the cells of the organism and the chemicals and low-level radiation to which they had been exposed. The defense repertoire exists at all levels of the biological hierarchy—from the molecular and biochemical level to the cellular and tissue level to the organ and organ system level. Cells contain preventive antioxidants to suppress oxidative damage to membranes. Cells also contain proteins and DNA; built-in redundancies for damaged molecules and organelles; tightly coupled redox systems; pools of reductants; antioxidants; DNA repair mechanisms and sensitive sensor molecules such as nuclear factor kappa beta; and signal transduction mechanisms affecting both transcription and post-translational modification of proteins needed to cope with oxidative stress. The biologic consequences of the low-level radiation that exceeds the background level of oxidative damage could be necrosis or apoptosis, cell proliferation, or cell differentiation. These effects are triggered by oxidative stress-induced signal transduction mechanisms—an epigenetic, not genotoxic, process. If the end points of cell proliferation, differentiation, or cell death are not seen at frequencies above background levels in an organism, it is unlikely that low-level radiation would play a role in the multistep processes of chronic diseases such as cancer. The mechanism linked to homeostatic regulation of proliferation and adaptive functions in a multicellular organism could provide protection of any one cell receiving deposited energy by the radiation tract through the sharing of reductants and by triggering apoptosis of target stem cells. Examples of the role of gap junctional intercellular communication in the adaptive response of cells and the bystander effect illustrate how the interaction of cells can modulate the effect of radiation on the single cell. — *Environ Health Perspect* 106(Suppl 1):331–339 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/331-339trosko/abstract.html*

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Evolution of Homeostatic Regulation of Multicellular Organisms in Hostile Environments

From Laszlo's analysis of living things as natural systems one may develop a view of man, in both the individual and social dimensions, as a hierarchy of such natural systems interconnected by various patterns of information flow in feedback circuits. "Health" may then be defined as the harmonious interaction of all hierarchical components, while "disease" is the result of a force which perturbs or disrupts hierarchical structure. The systems approach clarifies the relations among the biological and social sciences, points out the need for new concepts of medical practice, and suggests directions for closer contact between science and ethics. Further development of the systems view may yield significant results, both practical and theoretical (*1*).

One of the most difficult scientific and social/political issues to resolve today is: What are the biologic and health consequences to the human being and to society after low level exposures to radiation and toxic chemicals? Given that direct scientific data cannot provide the answers to these questions, many assumptions and extrapolated experimental and epidemiologic data currently are being used to derive various risk-assessment models. However, because our current understanding of the complex nature of the interactions of genetics, development, sex, diet, lifestyle, drug, and environmental pollutant factors into the induction of various chronic somatic diseases as well as hereditary diseases, it seems that the relevance of the risk-assessment models is scientifically questionable.

To derive a more biologically based risk-assessment model we will examine some of the relevant fundamental concepts in the evolution of multicellular organisms as related to the induction of various diseases by radiation and chemical toxicants. Life originated in a hostile environment. As primitive life forms evolved they did so in the presence of potentially harmful radiation and chemical toxicants. Ironically, humans as a species and as individuals evolved in an environment basically hostile to its very existence.

From the single fertilized egg through sexual maturation and reproduction to aging and death, the human being is not just approximately 100 trillion individual cells but a tightly orchestrated collection of different cell types (pluripotent stem cells, committed progenitor cells, and terminally differentiated cells) organized into tissues, organs, and organ systems and regulated by a cybernetic feedback of positive and negative signals (*1,2*). The normal external environment where this evolution, existence, survival, and reproductive success occurs contains a background level of both radiation and chemical toxicants.

For most organisms including humans, the energy for life is derived in an oxygenated atmosphere by oxidative metabolism that produces many potentially lethal reactive oxygen species (ROS) (*2,3*).

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Abbreviations used: GJIC, gap junctional intercellular communication; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; TPA, 12-*O*-tetradecanoylphonbol-13-acetate.

Together with background nonionizing and ionizing radiations as well as various chemicals that interact with the organism, additional ROS are generated. Single-cell organisms developed a series of protective mechanisms to respond to both exogenous and endogenously created potentially lethal toxicants. Clearly the maintenance of the genetic integrity for adaptive survival of the single cells, as well as the survival of the species, is paramount. Protection against DNA damage by various intracellular biochemical and DNA repair mechanisms (*4,5*) evolved to cope with the ambient and supra-ambient levels of physical and chemical toxicants. Both viable and lethal mutations can occur when the protective and repair mechanisms are breached. Death to both the single cell and the species could also occur by non-DNA damage; that is, by the destruction of subcellular organelles (e.g., membranes, mitochondria) by these toxicants (*6*).

The transition of the single-cell organism to the multicellular organism was accompanied by differentiation of cell types, which led to the development of various tissues, organs, and organ systems. In other words organization of single cells into collections of cells allowed the emergence of new protective and repair mechanisms at the cellular, tissue, organ, and system levels not found in the single cellular organism. Whereas the single-cell organism survived by its ability to proliferate, the multicellular organism survived and proliferated (e.g., in wound healing and redundancy of cells in tissues) by having differentiated functions and structures at all system levels and with adaptive responses of the genome to stress-induced signal transduction mechanisms (*7–10*).

Homeostatic control of cell proliferation, differentiation, and adaptive response of differentiated cells in multicellular organisms is dependent on three forms of communication: extra junctional, intra junctional, and gap junctional intercellular communication (GJIC) (*11*) (Figure 1). Whereas single-cell organisms respond to extracellular signals that trigger various intracellular signals (signal transducing mechanisms), the multicellular organism has the additional ability to homeostatically control cells coupled together by the membrane-associated protein channel gap junction (*12,13*). It is through gap junction channels that ions and low-molecular-weight molecules can freely pass, thereby allowing an equilibration of regulatory ions and molecules (*14*). Gap junctions can serve as sinks or point sources for critical cellular molecules (*15*).

The absence of gap junctions in single-cell organisms, and their presence in multicellular organisms, coincides with the evolutionary difference in the abilities of unicellular and multicellular organisms to survive and adapt. Specifically it has been speculated that the emergence of higher order biologic levels of cellular organization (e.g., tissues, organs, organ systems) and their homeostatic control of cell proliferation, differentiation, development, wound repair, and adaptive responses of differentiated cells was dependent on the appearance of the gap junction gene (*16*). In other words, the evolution of differentiation was causally related to the evolution of the gap junction gene. A family of highly evolutionarily conserved gap junction genes has been identified (*13*). Strong circumstantial evidence links the presence of functional gap junctions with growth control and differentiation (*12*).

If these gap junctions do play a major role in homeostatic regulation of cells in a multicellular organism and if homeostasis is the mechanism regulating health of the organism, then the question of risks with low-level exposures to various toxicants must involve an examination of how low-level exposures might affect GJIC-dependent homeostasis (*17,18*).

Role of Radiation and Chemical-induced Oxidative Stress on Homeostatic Regulation in Multicellular Organisms

All living organisms are threatened by the use of oxidative metabolism to generate energy because of the potentially lethal reactive oxygen metabolites and inescapable exposure to radiation and exogenous chemicals capable of generating ROS. Various mechanisms have evolved in unicellular and multicellular organisms to cope with ambient (background) and induced levels of oxidative stress. In unicellular organisms the single cell must cope with potentially lethal levels of oxidative stress-induced changes. The single cell of the unicellular organism is the unit of life. However, the unit of life or unit of function in a multicellular organism is really the syncytium of gap junctionally coupled cells (*19*).

In the multicellular organism the cells have developed various redox systems as well as compartmentalization and

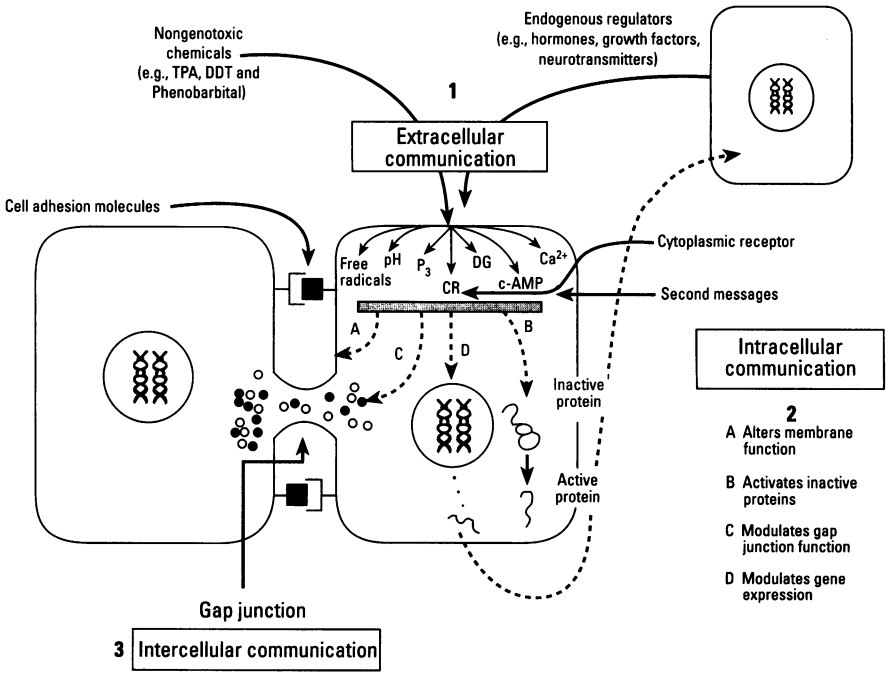

Figure 1. Heuristic schema characterizing the postulated link between extracellular communication and intercellular communication via various intracellular transmembrane signaling mechanisms. This framework provides an integrated view of how the neuroendocrine–immune system (mind or brain and body connection) and other multisystem coordinations could occur. Although not shown here, activation or altered expression of various oncogenes and antioncogenes could also contribute to the regulation of gap junction function. Reproduced from Trosko et al. (*95*), by permission of Elsevier Science Ireland Ltd.

damping strategies to cope with the inevitable generation of ROS.

To understand how low-level exposures of radiation or chemicals might affect multicellular organisms, several concepts must be considered. Photons or chemicals interact at the atomic or molecular levels of multicellular organisms (*20*). The events that occur within a cell after exposure to radiation and chemical toxicants may affect any number of molecules and/or organelles. Depending on the protective mechanisms of the organisms (e.g., melanin, drug-metabolizing enzymes, glutathione and antioxidant levels, redundancy of organelles such as multiple mitochondrion), and repair mechanisms, i.e., DNA excision repair enzymes, exposures might have little probability of disrupting the normal function. Moreover the type of cell at the time of exposure must be considered. Totipotent or pluripotent stem cells, committed progenitor cells, and terminally differentiated cells will respond differently to the same exposure because the state of differentiation will provide varying degrees of protection and repair. The biologic consequences of exposures of these cells to various toxicants will be different. For example if a pluripotent stem cell of a given tissue is hypersensitive to radiation, as is noted for murine small intestinal stem cells when compared to the stem cells of the large intestine (*21*), then differential oncogenesis might be expected.

If gene or chromosomal mutation frequencies in cells that survive radiation or chemical carcinogenic exposure are different in stem cells when compared to their differentiated daughter cells because of the differential apoptosis or necrosis rates of these cell types (*22*), then using *in vitro* genotoxicity assays without recognizing these confounding influences will lead to misleading risk assessments.

The impact of radiation or chemicals must exceed a background level to which the cells, tissues, organs, and organism normally have evolved and developed. Natural oxidative metabolism for life and natural exposures to background levels of radiations and toxicants in all environments create the noise levels that set the natural life span and disease spectra for each species. Artificial experimental control of the normal dietary habits of rodents, for example via caloric restriction, can dramatically affect both life span and disease spectra (*23,24*). If low-level exposure to particular radiations or chemical toxicants does not induce molecular events that exceed background levels in a multicellular organism, the biological risk will be canceled out by the normal risks of living.

The chain of events within the cell after exposure to low levels of radiation and chemicals permits oxidative reactions to occur. If such events exceed the normal capacity of the cell to quench these chemical reactions, a number of signal transduction mechanisms can be triggered (*18,19*). Depending on the type, cycle, and cell proliferative state (apoptosis or necrosis), differentiation or adaptive responses are possible biologic consequences of oxidative stress-induced signal transduction. With oxidative stress and the biologic response of the cell in a multicellular organism, both transcriptional and post-translational modification of gene expression (*25–28*) and modulation of GJIC can occur (*29,30*).

The prevailing paradigm assumes that all biologic consequences of low-level exposures to radiation and toxic chemicals are genotoxic because at higher doses these same agents can induce genetic damage in some cases (linear–no threshold dose–response model). However, current evidence indicates epigenetic mechanisms are induced by these agents. Ultraviolet ionizing radiation and chemical mutagens and carcinogens can induce gene expression (*8,31–35*), modulate cell–cell communication (*36,37*), cause apoptosis without DNA damage (*38*), and effect transformation *in vitro* (*39*) or carcinogenesis *in vivo* without genotoxicity (*40*). Even in cases where DNA lesions can be detected by one technique or another, mutations measured in some short-term assay, or mutations detected in oncogenes found in tumors formed after exposure to a chemical carcinogen, questions remain as to whether these lesions and mutations are related to the exposure (*41*).

No One Thing Causes Cancer: Carcinogenesis as a Multistep Multimechanism Process

As the age of the median life span has increased in developed countries, it has been estimated that one out of four individuals will develop some type of cancer before death. All of us are exposed from conception to both physical and chemical agents that can contribute to the carcinogenic process (a background level plus any increment above background because of genetic sensitivity differences, lifestyle differences, or accidental, deliberate, or unknowing exposures to physical and/or chemical agents). Therefore, the question remains as to how these exposures, both long-term low-level chronic exposures and acute higher levels, might contribute to the ultimate appearance of cancer.

Several concepts of the carcinogenic process must be integrated, as currently no one theory seems complete (*11*). The stem cell theory (cancer as a disease of differentiation or oncogeny as partially blocked ontogeny), the initiation/promotion/progression theory, the oncogene/tumor suppression theory, and the hierarchical/cybernetic theories were all derived by a number of unique empirical observations related to cancer. For example: *a*) not all cells in an organism seem able to give rise to neoplastic cells (*42*); *b*) cells of a given tumor appear monoclonal in origin (*43*); *c*) cells of tumors display varying degrees of differentiation without being able to terminally differentiate (*44*); *d*) tumor cells do not contact inhibit or do not have normal growth control (*45*); *e*) tumor cells have activated oncogenes and dysfunctional tumor suppressor genes (*46*); and *f*) tumor cells do not have normal positive or negative growth-regulator responses (*47*).

Recognizing that universal acceptance of each of these views of carcinogenesis is not a given [see stem cell theory vs the theory of dedifferentiation (*48*)], we assume that weight of the evidence gives substantial credibility to each theory but that no one theory can explain all of the empirical evidence related to the carcinogenic process or cancer phenotypes. Therefore, assuming each of the aforementioned theories is based on solid empirical and experimental data with regard to either the phenotypes of cancer cells or the carcinogenic process, it seems that GJIC could be the integrating factor that ties all of these incomplete theories together.

The carcinogenic process appears to involve the evolution of a pluripotent stem cell, which has the ability to terminally differentiate and proliferate and maintain its stemness, into a cell that starts to differentiate but is partially blocked during the differentiation process (Figure 2). This block might be considered the initiation phase. That is, the first step of the carcinogenic process prevents the mortalization or terminal differentiation of a stem cell (*49*). The concept runs counter to the prevailing idea that the early step of carcinogenesis involves the immortalization of a normal mortal cell (*50*). The partially differentiated initiated stem cell still has the ability to proliferate. Experimental evidence with

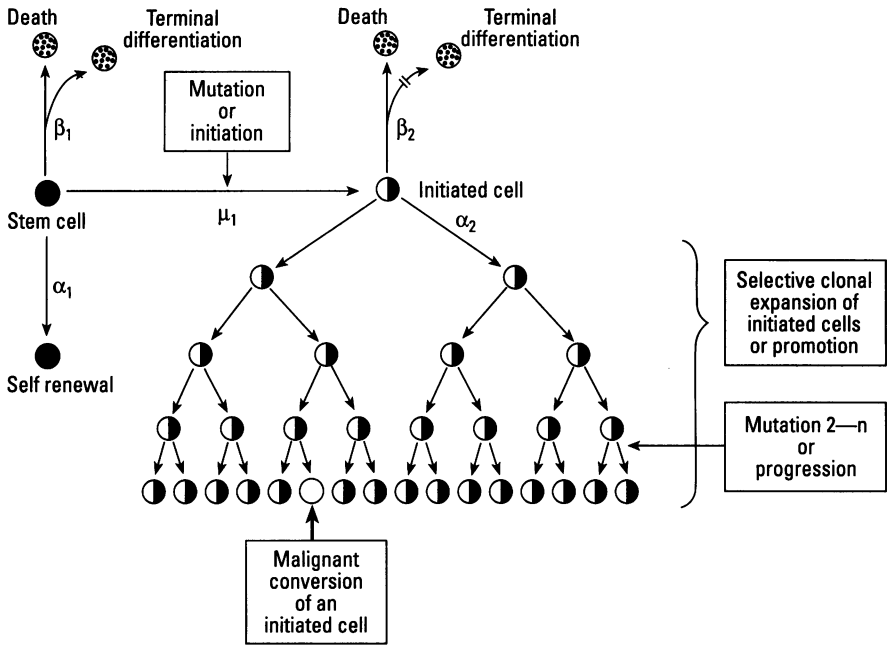

Figure 2. The initiation/promotion/progression model of carcinogenesis. β_1, rate of terminal differentiation and death of stem cell; β_2, rate of death but not of terminal differentiation of the initiated cell; α_1, rate of cell division of stem cells; α_2, rate of cell division of initiated cells; μ_1, rate of the molecular event leading to initiation (i.e., possibly mutation); μ_2, rate at which second event occurs within an initiated cell. Reproduced from Trosko et al. (*95*), by permission of John Wiley and Sons, Inc.

mouse skin cells is consistent with such a hypothesis. The observations of Nakano et al. (*42*), that only a small subset of cells in Syrian hamster embryos is able to be neoplastically transformed, also is consistent with the hypothesis. Moreover, stem-like cells have recently been isolated from normal human kidney (*51*) and breast epithelial tissue (*52*).

During the evolution of the initiated cell to a malignant, invasive, and metastatic tumor, the cells of the tumor acquire the phenotypes of malignancy (*12,53*). Whereas cancers usually contain cells with many different abnormal genotypes (an indication of genomic instability), evidence indicates that these cells had a clonal origin (*43*).

One can infer from the tumor promotion studies on liver, skin, bladder, and breast tissues that the single initiate stem cell is growth suppressed by either extracellular communication negative growth regulators such as transforming growth factor beta (TGF-β), and/or GJIC or both. The rationale for this idea comes from observations that animals initiated with a subthreshold dose of a carcinogen can live their lives without developing any tumors. Only after chronic and regular exposure to noninitiating tumor promoters can latent tumors appear (*54*). Most tumor promoters and tumor-promoting conditions (i.e., wounding, cell-killing compensatory mechanisms) inhibit GJIC (*55*) and block apoptosis (*56,57*). Inhibition in GJIC would allow the initiated stem cell to escape the suppressing effects of surrounding normal cells (*58*). By starting to differentiate, these initiated stem cells could be expressing specific gap junctions more closely related to the early stages of differentiation. Therefore, they are able to communicate with themselves (homologous communication) but not with the surrounding normal cells that express another connexin gene and are communicating homologously but not heterologously with the initiated cell. The fact that these benign initiated cells have some growth control via homologous GJIC could result from communicating with the normal cells by extracellular negative growth regulators such as hormones or TGF-β-like factors.

Tumor promoters can both inhibit GJIC (*58*) and modulate growth factor receptors (*59*) as well as trigger various intracellular communication or signal transduction systems (*60*). As long as gap junctions are downregulated by exogenous tumor-promoting chemicals or mitogenic endogenous growth factors and hormones, the initiated cells can proliferate. These clones of abnormal partially differentiated cells form the nodules of the breast, enzyme-altered foci of the liver, papillomas of the skin, and polyps of the colon. During the clonal expansion process caused by the tumor-promoting effect of downregulating GJIC and extracellular communication, if other changes occur that downregulate the function of gap junctions stably (e.g., activation of some oncogene, deactivation of a tumor-suppressor gene, mutation of extracellular regulators of gap junctions such as TGF-β, or mutation of the gap junction gene), then an exogenous or endogenous source of an inhibitor of GJIC would no longer be needed. Even the genomic dysfunction of cell adhesion molecules or extracellular matrix molecules would prevent GJIC even in the absence of any dysfunction of gap junction genes and their regulators. Cells that do not adhere do not have GJIC.

The proposed role of GJIC in carcinogenesis might explain the multistep nature of the process and why it seems easier to induce leukemias than solid tumors. In the former case the progenitor cells in the lympho-reticular system downregulate the gap junctions during normal differentiation to produce free-existing, differentiated cells. In other words normal differentiation eliminates one of the two major mitotic suppressing mechanisms that exist. These lympho-reticular free-existing progenitor cells can still communicate through extracellular positive and negative signals (e.g., cytokines, interferons, interleukins, etc.). Normal stem cells communicate with their differentiated daughters by extracellular growth regulators (*61–65*), whereas their differentiated progenitors communicate by gap junctions and extracellular growth regulators. Therefore to escape mitotic suppression both of these systems must be downregulated by either or both mutagenic or stable epigenetic mechanisms.

Evidence in support of this hypothesis exists. Most, if not all, cancer cells are characterized by dysfunctional homologous or heterologous communication. Most, if not all, tumor-promoting chemicals inhibit GJIC reversibly. Growth factors and hormones, which act as tumor promoters, inhibit GJIC. Oncogenes such as *ras*, *raf*, *neu*, *mos*, and *src* can downregulate GJIC. Tumor-suppressor genes can upregulate GJIC. Tumor cells transfected with various connexin genes can have their GJIC and growth regulation restored. Antisense connexin genes can downregulate GJIC and antitumor promoters and chemopreventive chemicals can upregulate GJIC (*11*).

The broad and integrating role of gap junctions in maintaining homeostatic control and acting as a mechanistic link in a systems model of human health is illustrated in three human genetic syndromes: Charcot-Marie-Tooth syndrome, Visceroatrial Heterotaxia syndrome, and mucoepithelial dysplasia syndrome (*66–68*). In addition, gap junction dysfunction has been linked to many disease states such as arrhythmias, cataracts, hypertension, and birth defects (*69–75*). In fact many chemicals that modulate GJIC have been associated with teratogenesis and neuro-, reproductive, and immune toxicities (*36*). Although these generalizations might seem implausible, one must remember that gap junctions are found in all tissues of the multicellular organism and that virtually every intracellular regulatory ion/molecule, as well as many signal transduction systems, have been involved in the up- or downregulation of GJIC. Gap junction genes and proteins have become very sensitive to internal and external factors that alter the steady-state oxidative metabolism of the cell. Perturbations of that background level could cause GJIC-mediated regulation of cell proliferation, differentiation, apoptosis, and adaptive responses of differentiated cells to change.

Therefore, from the standpoint of the initiation/promotion/progression theory of carcinogenesis, the dysfunction of GJIC plays a role primarily during promotion and progression phases. Downregulation by exogenous chemical promoters or endogenous growth factors and hormones must *a*) occur after a threshold level has been exceeded (*76–78*), *b*) be sustained in a regular and chronic fashion (*79*), and *c*) be potentially interrupted or reversed by chemicals that prevent downregulation or actually induce upregulation of GJIC (*11*) or by the absence of the tumor promoter. If and when stable downregulation of GJIC occurs by mutation in the connexin genes or the genes regulating the connexins or in the epigenetic transcription of these genes, an exogenous or endogenous source of tumor-promoting agents is no longer needed.

In summary, when we want to know if a given agent is a carcinogen we must ask if the agent initiates, promotes, or causes the stable conversion or progression of the tumor cell. If the agent initiates it must stably prevent a stemlike cell from terminally differentiating without blocking its ability to proliferate. Whether initiators can induce stable blockage of terminal differentiation of stemlike cells without exceeding a threshold level of exposure is not known. The stemlike cells must be mitotically regulated, and in the case of cells from solid tissues they must have GJIC. Immortal stemlike cells have been prevented from mortalizing or terminally differentiating by the initiation process. If the given carcinogen is unable to stably initiate cells (i.e., it is not a genotoxicant or mutagen) but is able to bring about the selective proliferation of the initiated cell, then the carcinogen acts as a tumor promoter by triggering a signal-transducing pathway in cells after the carcinogen exceeds a threshold or background level. The organism or initiated cell must be exposed to the promoting condition in a sustained fashion for a long period of time in the absence of antipromoting agents. If a given carcinogen can convert a preexisting benign tumor cell to become stably independent of exogenous or endogenous promoters such that the cell no longer has functional GJIC then the agent can be considered a progressor. Mutagens probably would be initiators. Epigenetic agents would be considered promoters and antipromoters depending on whether they down- or upregulate GJIC. Conceivably both mutagens and epigenetic agents that could bring about the stable downregulation of GJIC would be progressors. With genetic, developmental, and sex-related factors as potential modulators of how diet, workplace, lifestyle, and pollutants affect each of the phases of carcinogenesis, it is impossible to predict accurately on an individual basis the risk associated with exposure to any given agent. Moreover, realistically, as exposure is never by a single agent, additivity, antagonisms, or synergisms of effects could occur (*80,81*).

Risks to Chronic Low-level Exposures to Radiation and Chemicals: Thresholds, Adaptive Responses, and Pre- and Postmodifying Factors

Although exposures to acute or chronic high levels of radiation and chemicals in experimental animal or epidemiologic studies have yielded information regarding the potential of these agents to cause various human diseases and the mechanisms by which they might be induced, extrapolation to low-level chronic exposures has been hampered by the inability either to conduct statistically significant experiments or to collect sufficient epidemiological data. Consequently, models have been generated that assume a linear, no-threshold relationship between exposure to carcinogens and the disease end point. At the same time several challenges, both theoretical and experimental, have been made that are based on the assumption that thresholds and molecular/biochemical, cellular, and physiological mechanisms exist that are not consistent with a one-hit model for the actions of mutagens, cytotoxicants, or epigenetic agents to determine if the agent is a mutagen or not. The challenge is not trivial (*41*).

If we wish to determine what might happen to an organism when it is exposed to a low level of a carcinogen, the terms level and carcinogen must be understood. Implicit in such terms are the concepts of thresholds or linear responses to the agent of concern and of initiators, promoters, and progressor (genotoxic, cytotoxic, and epigenetic) agents. Clearly all organisms are chronically exposed to low level or background levels of potential carcinogenic initiators and promoters. As discussed earlier normal organisms have evolved adaptive mechanisms to cope with this background noise. Therefore low-level exposure implies that the level of exposure that is above the background level does not easily lead to a causally linked biological or disease end point but could induce a molecular, cellular, or physiologic response. The implication is that the multicellular organism can adaptively respond to signals induced by physical and chemical agents without incurring any irreversible or irreparable damage.

The many levels of biologic organizations in the hierarchical systems of a multicellular organism provide at least a no-effect threshold level after low-level exposures. For example, an ionizing tract could pass through a cell without inducing DNA damage or even inducing an epigenetic change. Depending on the cell the ionizing tract that exceeds intracellular protective barriers might still not make a biologic impact that would lead to an end point of disease. Specific DNA damage could be repaired in an error-free manner. The damage that is not repaired could lead to cell death or mutation in a stem or progenitor cell. The same ionizing tract could also alter the redox state of a cell. The induced oxidative stress could trigger signal transduction mechanisms that, depending on the state of the cell or the cell type, might activate genes for differentiation, cell proliferation, or apoptosis.

Depending on when this low-level exposure occurs in the course of development (e.g., embryogenesis, organogenesis, neonate, adolescence, adult, or aging adult

stages), the impact of a gene or chromosomal mutation or the death of a cell or an activated gene also depends on the type of cell in which these events occur and on subsequent exposures. In theory a single low-level exposure could set the stage for a subsequent reaction of the cell to another low-level exposure or a higher level exposure. In theory another exposure could cause an additive, synergistic, or antagonistic effect at the cell level. Additionally, the hierarchical strata above the cell level could determine consequences at the organism level.

To date there has been experimental and epidemiologic evidence that supports an adaptive response or hormesis (*82,83*) on both the cell and organism levels. In other words cells *in vitro* or organisms exposed to low levels of a physical or chemical agent had less biologic damage with subsequent higher level exposure than cells or organisms that received the same total dose given acutely. At each level of organization there are possible explanations for such findings. For example, low-level exposure could induce signal transduction to activate DNA repair enzymes, glutathione synthetase, and other protective or repair mechanisms so that subsequent exposure to higher levels of a genotoxicant or cytotoxicant positions the cell to cope more effectively with any possible lethal or mutagenic lesion. Whether the adaptive response on the cell level impacts on the next higher level would depend on the cell in which the adaptive response was noted, whether the adaptive response actually prevented any stable mutagenic or epigenetic event to occur in the surviving cell, and whether the cell rescued by the adaptive response actually could be clonally multiplied. An abnormal cell such as a carcinogenic initiated stem cell will have a chance to effect a pathologic or physiologic change only if it is multiplied.

At the cellular level there is evidence that the adaptive response is mediated by GJIC (*84*). Older literature provides observations that cells irradiated in spheroids are more radioresistant than those in two-dimensional contact inhibited environments or in sparse *in vitro* conditions (*17*). As antioxidants such as glutathione can pass through gap junctions (*85,86*), and because glutathione status of cells has been correlated with radiation and drug sensitivity (*29,30,87–89*), cells that are gap junctionally coupled or uncoupled could respond to radiation exposures or chemical toxicants. Only a few studies have been performed on the role of low levels of ionizing radiation on gap junctions (*37*). It appears, at least at doses below 0.5 Gy, that the structure of gap junctions is normal. Functionality of the gap junctions below 0.5 Gy has not yet been determined. If radiation, a potential genotoxicant and cytotoxicant, does not inhibit gap junctions at doses below 0.5 Gy, then gap junctions could provide the channels by which important antioxidants could aid in the rescue of the damaged cell or through which the death signal could pass to induce apoptosis (*57*).

If the chemical to which the organism is exposed at the cellular level is not a genotoxicant but is associated with the appearance of tumors in rodent assays, it is probably a cytotoxicant such as chloroform or an epigenetic agent such as TCDD, phenobarbital, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), or polychlorinated biphenyls. These chemicals can all induce oxidative stress, activate various signal transducing mechanisms, and modulate GJIC. Most importantly, in the context of low-level exposures all known epigenetic and nongenotoxic cytotoxicants have threshold levels of effects (*36,37,76*). In other words, a multicellular organism must be exposed to a high enough level of the nongenotoxicant before the agent will act as a tumor promoter. The same agents at the cellular level also exhibit threshold levels before they modulate GJIC or kill cells (*36,90*). The significance of this is that just because one can detect some molecular and biochemical or even cellular response to a potentially toxic physical agent at a low level, it does not necessarily follow that there will be a biologic or disease consequence. In other words below the threshold levels at which nongenotoxic cytotoxicants can kill cells or epigenetic agents can modulate GJIC, the cell will restore the redox state and various cybernetic systems will bring the cell back to ground state. Clearly each chemical of this class has its own unique threshold at which it can modulate GJIC or kill cells (e.g., TPA, DDT, saccharin) and the species or cell type will respond differently to the same chemical. Moreover, to complicate the discussion but put these generalizations into biologic context, mixtures of this class of nongenotoxic chemicals can either exhibit additive, antagonistic, or synergistic interactions at both the cellular and whole-organism level (*80,81,91*).

Usually a sustained chronic exposure to nongenotoxic chemicals at threshold levels and above must be maintained in order for the appearance of a disease state such as cancer to manifest itself (*79*). Exceptions to this generalization would be the acute exposure to a threshold level of a given nongenotoxic agent or to a mixture of nongenotoxicants at a critical period of embryonic or fetal development (*92*). Alteration of the adaptive function of differentiated cells by nongenotoxic agents at critical times in organogenesis and development could lead to teratogenesis (*90,92,93*).

Implicit in the use of the term low-level exposure is the idea that the adaptive trigger must be that which exceeds the background level of oxidative metabolic and oxidative stress of the cells of the organism, but not high enough to cause negative consequences to the cell or organisms. The exact dose and the timing between the adaptive exposure and the subsequent damaging dose appear to depend on the system being studied. It is possible that a low dose of ionizing radiation could induce transcription of genes needed to protect the cells from subsequent exposure to higher doses by inducing an increment of oxidative stress above normal background levels. It is possible that the induction of antioxidants, reductants, or cell-cycle checkpoint inhibitors to facilitate time for DNA repair could provide a mechanism to explain some of the reported adaptive responses. GJIC has been proposed as a mediator of the adaptive response (*94*). Ishii and Watanabe (*84*) reported recently that GJIC appears necessary to see an adaptive response after X-ray exposure.

In summary the absorption of radiation's energy by molecules in stem, progenitor, and terminally differentiated cells in a multicellular organism must overcome multiple barriers built into cells, tissues, organs, and organ systems in order to alter homeostatic control of cell proliferation, differentiation, wound healing, apoptosis, and adaptive responses of differentiated cells. If the frequency of these end points exceeds the normal background levels in specific tissues after exposure, particularly in normal and/or initiated stem cells, such exposure could result in disease.

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